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New Composite Biocarriers Engineered To Contain Adsorptive and Ion-Exchange Properties Improve Immobilized-Cell Bioreactor Process Dependability

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Novel biocarriers that combine the adsorptive properties of activated carbon with the ion-exchange properties of zeolite-based Type Z inorganic oxide biocarriers (D. R. Durham, L. C. Marshall, J. G. Miller, and A. B. Chmurny, Appl. Environ, Microbiol. 60:3329–3335, 1994.) were developed. These biocarriers, designated Type CZ, possess fundamental properties that heretofore have not been described for available microbial immobilization matrices. Type CZ biocarriers provide an environment that promotes dense microbial colonization and maintains bioreactor productivity by buffering immobilized microorganisms from unfavorable operating conditions. Data demonstrating protection of immobilized bacteria from organic shock loads and extended pH shocks are presented. In addition, bioreactors containing the composite Type CZ biocarriers continue to remove waste stream contaminants during periods of oxygen deprivation and nutrient limitation.

Immobilized-cell bioreactor technology utilizes the colonization of specialized microorganisms onto inorganic surfaces as fixed films for the degradation of chemical pollutants in environmentally controlled bioreactors (3, 7, 8). The advantages of this technology over conventional suspended-cell growth systems are well documented (3, 6, 8, 9, 14). Recently, we described a biocarrier, Type Z, that provides a porous, high-surface-area matrix supporting dense colonization with microorganisms (3). These biocarriers exhibit ion-exchange properties that buffer microbial populations from acid and base pH system shocks and nutrient limitation and enable immobilized microorganisms to rapidly recover from conditions of oxygen deficiency and organic system overloads. The Type Z biocarrier, however, does not provide adequate protection from toxic organic shock loads. The adsorptive properties of granular activated carbon (GAC), on the other hand, have been demonstrated to protect immobilized microorganisms from excessive fluctuations in concentrations of organic compounds (5–7, 9). In addition, GAC has been shown to enhance biodegradation of low-strength wastes by concentrating the organic compounds at the liquid-solid interface (11, 15) and to adsorb inhibitory or recalcitrant compounds (5, 10). The microporous surface of GAC precludes internal accessibility to microorganisms (10, 11), however, and its low mechanical strength may make its use as a biocarrier problematic in many full-scale biotreatment processes.

In this communication, we describe modified Type Z biocarriers that contain activated carbon. These new composite extrudates exhibit buffering properties towards a variety of process upsets. Laboratory-scale results indicate that these biocarriers confer the advantages of activated carbon as an adsorbent as well as the ion-exchange characteristics and colonization potential of the Type Z matrix.

Bioreactors (2.1-liter Kontes columns) were packed with various biocarriers and colonized with a strain of *Pseudomonas putida*, WRC-9, which was isolated from activated sludge by enrichment culture with *N*-methyl-2-pyrrolidone (NMP) as a sole carbon and nitrogen source. The bioreactors were run in an up-flow mode as previously described (3), with a hydraulic retention time of 20 h. The reactors received a common influent feed containing basal salts medium (BSM) (4) with 1/10 the normal nitrogen concentration and 3,000 ppm of NMP. Degradation of NMP was monitored by high-performance liquid chromatography (HPLC) as described elsewhere (3). In all cases, reactors were operated for a sufficient period of time to allow the microbial population to reach steady-state kinetics. Microbial biomass was established for more than 1 month before experimentation was initiated. These bioreactor systems were maintained for extended periods of time (9 to 12 months) and were used for the duration of this study.

A comparison of the physical properties of Type CZ and Type Z biocarriers is shown in Table 1. The Type CZ extrudate is a composite of Type Z biocarrier (silica alumina matrix containing a zeolite molecular sieve) and activated carbon (5% by weight). This product is extruded as 1/4-in. (1 in. = 2.54 cm) pellets with a 1:1 aspect ratio and is similar to the Type Z matrix with respect to mean pore diameter, accessible surface area and pore volume for microbial colonization, and ion-exchange capacity (Table 1). Although activated carbon can be incorporated to concentrations of 15%, increasing concentrations reduced the mechanical strength as well as the pore volume accessible for microbial colonization (unpublished data).

Evaluation of the Type CZ biocarrier revealed that these surfaces were colonized to 10^9 viable microorganisms per g of carrier (ATP analysis) (2, 3). Comparative volumetric productivities of bioreactors containing various biocarriers colonized with *P. putida* WRC-9 were determined and did not show substantial differences between bioreactors containing Type CZ biocarriers and those containing other porous surfaces. Previously, it was demonstrated that this is not the case for comparisons to nonporous biocarriers. For example, bioreactors containing Type Z biocarriers were 4.5-fold-more proficient for removing phenol from a waste stream than were those containing plastic surfaces (3). The data indicate that the Type CZ bioreactor productivity (1.52 kg of NMP degraded per m^3 per day) was comparable to that of reactors containing the Type Z biocarriers (1.42 kg of NMP degraded per m^3 per day).

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TABLE 1. Comparative properties of Type CZ and Type Z (3) biocarriers

Biocarrier	Mean pore diam (μm)	Surface area (m^2/g)[a]	Accessible surface area (m^2/g)[b]	Pore vol (ml/g)		Crush strength (lb/in^2)	Exchange capacity (meq/g)	Carbon concn (%)	Colonization (CFU/g)[c]
				Total[a]	Accessible[b]				
Type CZ	8	78	0.14	0.52	0.19	19	0.36	5–15	10^9
Type Z	7–10	79	0.14–0.17	0.53	0.2–0.24	20	0.4	0	10^9

[a] Determined by mercury porosimetry (13).
[b] Determined on the basis of a pore diameter greater than 4 μm.
[c] Determined by ATP analysis as previously described (2, 3).

The Type CZ bioreactor was similar in performance to a bioreactor containing pumice (1.40 kg of NMP degraded per m^3 per day) as the biocarrier but was more than 20% more efficient (1.18 kg/m^3/day) than a bioreactor containing GAC (Filtasorb F-300; Calgon).

The advantages of the composite Type CZ biocarrier are most apparent when the bioreactors are subjected to adverse operating conditions or system shocks. In general, immobilized microorganisms are more tolerant to process upsets than suspended-cell systems such as activated sludge (3, 5, 9). The control of shock loads of organic pollutants during biotreatment has been cited as one of the most critical factors for the successful operation of these processes (5, 14). The results shown in Fig. 1 demonstrate the effect of an organic system shock of phenol to bioreactors containing NMP-degrading bacteria immobilized onto adsorptive and nonadsorptive biocarriers. Immobilized *P. putida* WRC-9, which does not metabolize phenol and is sensitive to 200 ppm of phenol in shake flasks (data not shown), was subjected to consecutive phenol loadings of 15,000, 10,000 and 10,000 ppm as 1/10 column volumes. The bioreactor containing the Type CZ biocarrier adsorbed 97% of the phenolic surges (Fig. 1A), which protected the NMP-degrading population, and thus maintained reactor productivity (Fig. 1B). Similar results were obtained with the GAC bioreactor, but bioreactors with microorganism colonization on the nonadsorptive biocarriers, pumice or Type Z, exhibited substantial reductions in performance (Fig. 1). NMP degradation recovered to greater than 95% after phenol was diluted from these bioreactors. Phenol slowly desorbed from the Type CZ and GAC biocarriers (data not shown).

The adsorptive properties of the Type CZ composite biocarrier also assisted in maintaining reactor productivity during transient periods of oxygen starvation. For example, as depicted in Fig. 2, bioreactors containing these biocarriers demonstrated only a slight reduction in NMP removal during a 24-h oxygen deprivation experiment. Similar results were observed with GAC, whereas the nonadsorptive biocarriers showed a substantial decrease in NMP degradation. These results are consistent with the capacity of activated carbon to enrich surfaces with organic compounds (12), resulting in abiotic removal of NMP under conditions in which the immo-

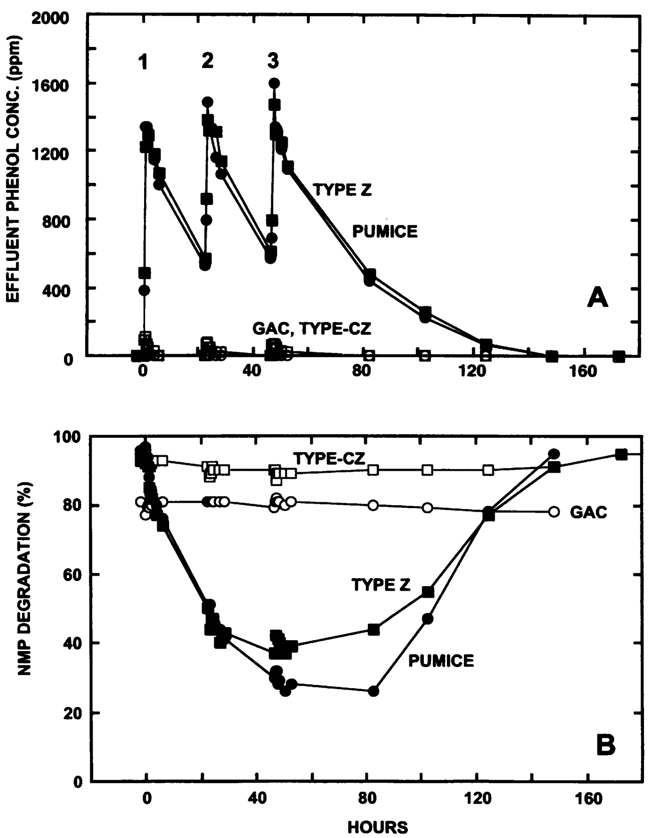

FIG. 1. Effect of phenol shock loads on bioreactor productivities. Bioreactors colonized with bacteria degrading a 3,000-ppm NMP feed were subjected to consecutive 15,000-, 10,000-, and 10,000-ppm spikes of phenol as 1/10 column volume additions (labeled 1, 2, and 3, respectively). The concentration of phenol (A) and the degradation of NMP (B) in the effluents were determined by HPLC (3). These data are representative of two independent experiments.

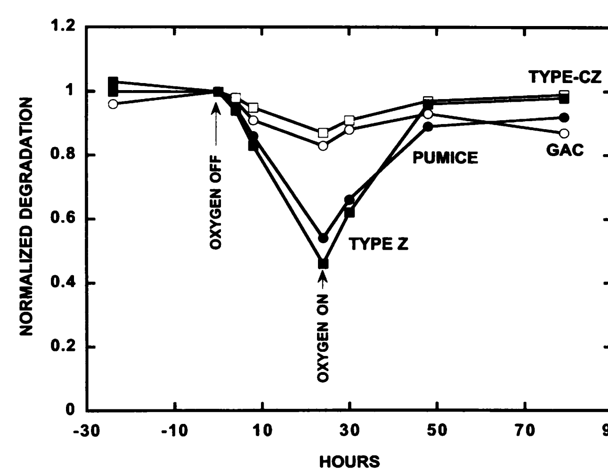

FIG. 2. Effect of oxygen deprivation on bioreactors containing immobilized microorganisms. Oxygenation was discontinued from the bioreactors for 24 h, and NMP removal was determined by HPLC (3). The data are indicative of four independent experiments.

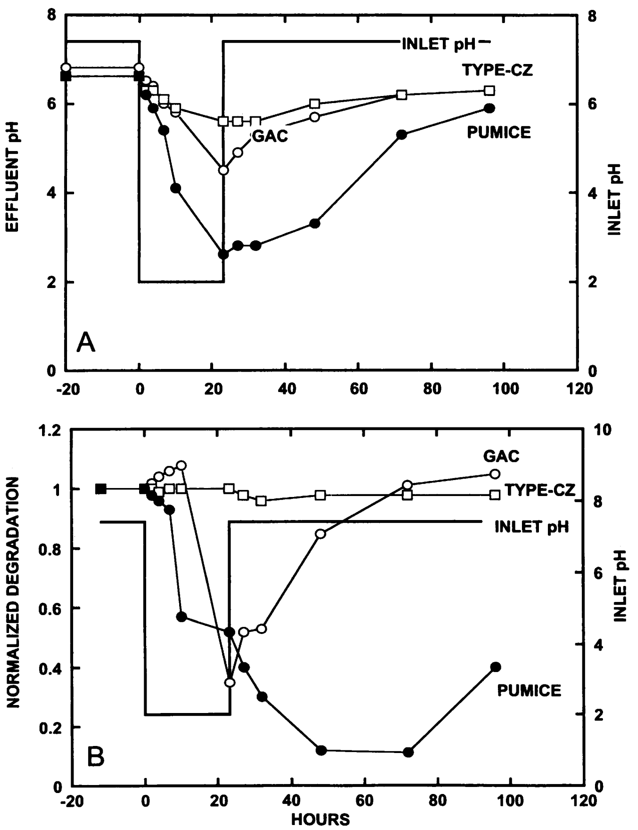

FIG. 3. Demonstration of acid shock resistance of bioreactors containing Type CZ biocarrier. Bioreactors were fed a common feed of NMP-BSM acidified to pH 2 with 18 mM sulfuric acid. The effluents were monitored for pH (A) and NMP degradation (B). The data are indicative of duplicate, independent experiments.

bilized population was unable to oxidize the influent contaminant.

Like Type Z biocarriers (3), the Type CZ composite biocarriers also possess ion-exchange properties (Table 1) that buffer bioreactor systems from aberrations in operating pH. The inherent buffering capabilities of these materials are due to the zeolite constituent, a class of microcrystalline aluminosilicate materials that have cation exchange properties (1, 3). Previously, it was shown that the Type Z biocarriers moderated acidic and basic pH changes under abiotic and colonized conditions (3). The effect of a decrease in waste stream pH on bioreactor performance is shown in Fig. 3. Bioreactors, containing either Type CZ composite biocarrier, GAC or pumice, received a common feed of NMP medium acidified to pH 2 with sulfuric acid for a period of 24 h (~1 column volume), after which the influent feed was returned to standard medium (pH 7.2). The bioreactor containing the Type CZ composite biocarrier exhibited a slight decline in effluent pH and no loss in reactor performance. In contrast, bioreactors containing either GAC or pumice demonstrated a significant reduction in effluent pH (Fig. 3A) and NMP degradation (Fig. 3B). In a separate experiment, bioreactors with zeolite-based materials, diatomaceous earth, and plastic packing materials were subjected to prolonged acid conditions (Fig. 4). Bioreactors containing microorganisms immobilized onto Type CZ or Type Z surfaces exhibited no loss in bioreactor productivities after receiving 14 column volumes of medium (pH 2) (Fig. 4).

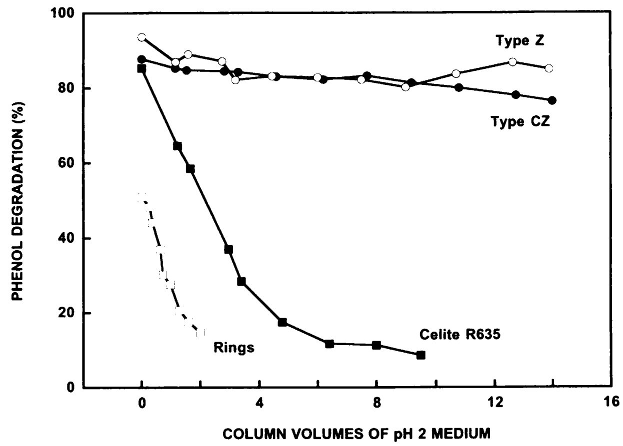

FIG. 4. Effect of prolonged acid exposure on bioreactor performance. Bioreactors containing various packing materials were colonized with *P. aeruginosa* (3) and received 14 column volumes of a phenol (1,000 ppm)-BSM feed (pH 2). Phenol degradation was determined by HPLC (3). The rings used were plastic 3/8-in. Flexirings (Koch Engineering), and Celite R635 is a diatomaceous earth biocarrier (Johns Manville).

Interestingly, there was an eventual reduction of effluent pH to pH 4, concomitant with a 1-log reduction of the immobilized population. The bioreactor volumetric productivities, however, remained constant throughout the duration of the experiment. The ion-exchange properties of the Type CZ and Type Z biocarriers also permitted microorganisms immobilized onto these matrices to continue to degrade organic compounds during exclusion of nitrogen from the influent feed for a 16-day experimental period without loss in reactor productivity (data not shown). Zeolite mediates the binding of ammonia, which serves as a source of reserve nitrogen, to the immobilized microorganisms (3). It is interesting to note that the ion-exchange and adsorptive properties exhibited with the Type CZ biocarrier were observed when the matrices were heavily colonized with microorganisms or covered with biofilm for extended periods.

In summary, biocarriers, containing both adsorptive and ion-exchange properties, that have the potential for improving the reliability of immobilized-cell bioreactor systems were developed. Collectively, the data presented herein indicate that these surfaces provide a suitable matrix for colonization, resulting in proficient volumetric productivities and unparalleled protection of immobilized microorganisms from adverse operating conditions. Thus, these new biocarriers may represent a major advancement for the full-scale biotreatment of aqueous wastes in industrial applications.

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